Theriogenology 86 (2016) 270–277

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Review article

Embryo development in dairy cattle

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ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 13 January 2016
Accepted 14 March 2016

Keywords:
Conceptus
Embryo mortality
Cattle
Embryo gene expression

ABSTRACT

During the past 50 years, the fertility of high-producing lactating dairy cows has decreased, associated with intensive selection for increased milk production. The physiological and metabolic changes associated with high milk production, including decreased (glucose, insulin, IGF-I) or increased (nonesterified fatty acids, ketone bodies) concentrations of circulating metabolites during nutrient partitioning associated with negative energy balance as well as uterine and nonuterine diseases have been linked with poor reproductive efficiency. Fertilization is typically above 80% and does not seem to be the principal factor responsible for the low fertility in dairy cows. However, early embryonic development is compromised in high-producing dairy cows, as observed by most embryonic losses occurring during the first 2 weeks after fertilization and may be linked to compromised oocyte quality due to a poor follicular microenvironment, suboptimal reproductive tract environment for the embryo, and/or inadequate maternal–embryonic communication. These and other factors related to embryo development will be discussed.

1. Introduction

In the modern Holstein, calving rates are close to 55% to 60% in heifers and 35% to 40% in lactating dairy cows. The physiological changes associated with high milk production have been linked with poor reproductive efficiency in commercial dairy herds [1]. Decreased (glucose, insulin, IGF-I) or increased (nonesterified fatty acids [NEFA], ketone bodies) concentrations of circulating metabolites during nutrient partitioning associated with low body condition score and disease status undoubtedly play a role in determining reproductive outcome. However, understanding the causes of infertility in dairy cattle is complex and may be attributable to impacts at numerous points along the developmental axis including compromised follicle development impacting on oocyte quality, a suboptimal reproductive tract environment incapable of supporting normal embryo development, or a combination of both [2].

Many reviews of dairy cow fertility in the past few decades have begun with reference to the antagonistic relationship between selection for milk production and cow fertility. It is clear that at herd level, there are negative genetic correlations between production and reproduction. Phenotypically, however, within a herd, individual high-producing cows are often more fertile, possibly a reflection of animal health [3]. Others have highlighted that the production–reproduction antagonism may not be as pervasive as generally believed [4,5]. Undoubtedly, cow fertility worldwide has declined over the past 50 years, whether measured in terms of calving interval, duration from calving to conception, or number of inseminations required for conception (pregnant/artificial insemination [AI]) [1]. Nonetheless, with changes in the indexes used for sire and dam selection, such as the Economic Breeding Index in Ireland, which include weightings for fertility

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0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.04.040

(calving interval), survivability, and health traits, the decline in fertility would appear to have been halted and perhaps even somewhat reversed [6].

Sartori et al. [7] recently comprehensively reviewed published data on fertilization and embryo quality (during the first week after conception) and the main factors responsible for the low fertility in single-ovulating and superovulated dairy cows. Rather than repeating this information, the focus of this review was to summarize recent data on embryo development in dairy cows with an emphasis on development after the first week, encompassing conceptus elongation and maternal recognition of pregnancy.

2. Subfertility—a multifactorial issue

Assuming cows are submitted for AI at an appropriate time or are bred naturally, reproductive failure results from fertilization failure or poor embryo survival after fertilization. Studies in dairy and beef cattle indicate that fertilization rates are typically high (>80%) and are higher for nulliparous dairy and beef heifers and nonlactating beef cows than lactating beef and dairy cows and nonlactating dairy cows. These data have been comprehensively reviewed by several authors [7–10].

2.1. Follicle/oocyte

Given that most embryonic loss occurs early during pregnancy, one can implicate several components of the early developmental continuum including the follicle, oocyte, embryo, and/or conceptus–uterine interaction.

In vitro studies examining the effect of lactation or genetic merit for milk production on oocyte quality have led to equivocal results. For example, Snijders et al. [11] found that a lower proportion of oocytes recovered from dairy cows with a higher genetic merit for milk production underwent cleavage or developed to the blastocyst stage *in vitro* compared with those from cows of average genetic merit. Rizos et al. [12] reported no difference in the proportion of good-quality oocytes undergoing fertilization and development to the blastocyst stage between lactating cows and heifers after ovum pick up/IVF. Several studies from Virginia [13–15] demonstrated that conditions related to early lactation have a negative effect on oocyte quality and endocrine measures in dairy cattle; however, in these articles, oocyte quality was assessed based solely on morphology, which may be of limited value. A more recent study from our group [16] involving ovum pick up in lactating dairy cows from 7 to 85 days postpartum failed to demonstrate an effect of metabolic status postpartum on oocyte ability to undergo IVF and develop to the blastocyst stage *in vitro* despite expected changes in circulating metabolites during the same period reflective of cows in negative energy balance. It may be that IVF, which typically is limited to blastocyst development rates of 30% to 40% from starting immature oocytes, is simply not sufficiently sensitive to detect subtle differences in oocyte quality.

Evidence supporting a contribution of poor oocyte quality to subfertility in dairy cows comes from a variety of sources. First, the limited available data on nonsurgical flushing of unstimulated dairy cows (reviewed by [7]) suggest that a significant proportion of embryos degenerate before the blastocyst stage. For example, in three studies by Cerri et al. [17–19], the proportion of viable embryos recovered on Days 6 to 7 was approximately 50%. Given that the fertilization rate is estimated at 85% to 95%, this suggests that a significant proportion of embryos are lost as early as Day 7. Second, several studies have reported higher pregnancy rates in lactating dairy cows after embryo transfer (ET) compared with AI [20–26]. However, it should be noted that many of these studies used cows exposed to heat stress where the oocyte and/or embryo are damaged by maternal hyperthermia [27]. If one examines studies in which heat stress was not a factor, the difference in pregnancy rates between AI and ET is less striking or absent [28,29]. Third, exposure of oocytes *in vitro* to NEFA at physiological concentrations consistent with those measured in the preovulatory follicle of postpartum lactating cows is detrimental to oocyte development [30–33] and oviduct cell function [34] and subsequent embryo development [35]. Furthermore, Girard et al. [36] reported that negative energy balance altered gene expression in granulosa cells of dairy cows at 60 days postpartum.

Consistent with these data, the metabolomic profile of follicular fluid (FF) from heifers and postpartum nonlactating and lactating cows has highlighted differences in the microenvironment in which the oocyte develops which may contribute to compromised oocyte quality [37–39]. Bender et al. [37] investigated the metabolic differences between FF from the dominant follicle of lactating cows and heifers using gas chromatography mass spectrometry–based metabolomics. FF and serum were collected from cows and heifers over three phases of follicle development: newly selected dominant follicles, preovulatory follicles before estrus, and post-LH surge follicles. Analysis of the fatty acids revealed 24 fatty acids and nine aqueous metabolites which were significantly different between cows and heifers. Of particular interest were the higher concentrations of saturated fatty acids (palmitic acid, stearic acid) in FF from cows and higher docosahexaenoic acid levels in FF from heifers.

Forde et al. [38] examined the effect of lactation on the composition of preovulatory FF in postpartum Holstein cows and maiden heifers. Principal component analysis of FF metabolites revealed a clear separation of lactating cows from both nonlactating cows and heifers. The amino acids tyrosine, phenylalanine, and valine and fatty acids heneicosanoic acid and docosahexaenoic acid were all lower in FF from lactating compared with dry cows. FF from lactating cows was higher in aminoadipic acid, α-aminobutyric acid, glycine, and serine, whereas histidine, leucine, lysine, methionine, and ornithine were all lower than in dry cows and heifers. The ratio of n6:n3 was higher in lactating cows compared with both nonlactating cows and heifers, whereas total n3 polyunsaturated fatty acids, pentadecanoic, linolenic, elaidic, and arachidonic acids were all lower in the FF of lactating cows than both nonlactating cows and heifers.

Using a previously established dairy cow fertility model [40,41], Moore et al. [39] examined the metabolomics

profile of FF from the first-wave dominant follicle of dairy cows with similar genetic merit for milk production but with extremes of good or poor genetic merit for fertility. The abundance of nine fatty acids (arachidic acid, heneicosanoic acid, myristic acid, behenic acid, myristoleic acid, heptadecenoic acid, cis-11-eicosanoic acid, nervonic acid, and γ-linolenic acid) in FF was affected by genotype. Concentrations of cysteine, leucine, ornithine, proline, and tyrosine in FF and asparagine, creatinine, cysteine, methionine, proline, and valine in serum were also affected by genotype. Authors concluded that FF and serum fatty acids and FF amino acids that were significantly affected by genotype were highly predictive of fertility genotype.

Others have attempted to correlate FF composition directly with oocyte developmental competence. For example, Matoba et al. [42] investigated the ability of a panel of follicular parameters including intrafollicular metabolomic profiles to predict the potential of bovine oocytes to develop to the blastocyst stage *in vitro*. Principal component analysis of the quantified aqueous metabolites in FF showed differences between oocytes that formed blastocysts and oocytes that degenerated; l-alanine, glycine, and l-glutamate were positively correlated, and urea was negatively correlated with blastocyst formation. FF associated with competent oocytes was significantly lower in palmitic acid and total fatty acids and significantly higher in linolenic acid than FF from incompetent oocytes. Sutton-McDowell et al. [43] related the composition of FF and blood plasma from individual Holstein dairy cows to the *in vitro* developmental competence of pooled abattoir-derived oocytes. Cumulus–oocyte complexes were matured in either 50% FF or 50% plasma. Blastocyst rates were negatively related to plasma glucose and days postpartum and positively related to body condition score and plasma NEFA levels. Total NEFA levels in FF did not influence oocyte developmental competence *in vitro*. Results suggest that days postpartum and body condition score influence carbohydrate metabolism within the follicular environment.

2.2. Reproductive tract environment

Consistent with the multifactorial nature of subfertility in dairy cows, the reproductive tract (oviduct/uterus) clearly also plays a crucial role in providing an appropriate environment conducive to normal embryo development leading up to maternal recognition of pregnancy, a period around which a substantial part of embryo loss occurs [9]. Several studies from our group [44–47] and others [48,49] have emphasized the important role of progesterone in the first week after conception in establishing an optimum uterine milieu to support conceptus elongation around the time of maternal recognition. ET studies allow us to test the ability of the reproductive tract to support development without the confounding effect of the cow's own, potentially compromised, oocyte. Studies from our group using multiple ETs of *in vitro* produced embryos indicate that the ability of the reproductive tract of postpartum lactating cows to sustain embryo development is compromised compared to nulliparous heifers [50] or postpartum nonlactating cows [51] between Days 2 and 7. The same trend was observed between Day 7 and Days 14 to 16 when lactating versus nonlactating cows are compared [52] but not when postpartum lactating versus postpartum dry cows were compared [51]. We reported that embryo development to Day 7 in the reproductive tract of postpartum lactating cows was compromised compared with that in the tract of nulliparous heifers [50], consistent with the data reviewed by Sartori et al. [7]. In that study, 100 two- to four-cell embryos were endoscopically transferred to the oviduct ipsilateral to the corpus luteum of nulliparous heifers or postpartum lactating cows on Day 2 after estrus. Five days later, on Day 7, the oviduct and uterus were flushed nonsurgically to recover the embryos. More embryos were recovered from heifers than cows (79 vs. 57%, respectively). Of the embryos recovered, 34% had developed to the blastocyst stage in the heifer oviduct, a typical yield given that immature oocytes from abattoir-derived ovaries were used, compared with only 18% in the postpartum cow oviduct.

One justifiable criticism of the model described previously is that a nulliparous heifer is not the same as a metabolically stressed postpartum cow in early lactation. To overcome this criticism, Maillo et al. [51] used age-matched postpartum primiparous dairy cows that were either milked postcalving (i.e., lactating) or were dried off immediately at calving (i.e., never milked, nonlactating) to directly test the effects of lactation on postpartum fertility characteristics. Lactation induced a significant alteration in the pattern of many key metabolites associated with fertility in postpartum cows which was associated with an impairment in the ability of the reproductive tract of the postpartum lactating dairy cow to support early embryo development to the blastocyst stage around Day 60 postpartum. Following endoscopic transfer of two- to four-cell *in vitro* produced embryos to the oviduct ipsilateral to the corpus luteum as described previously, more embryos had formed blastocysts in nonlactating compared to lactating cows (40 vs. 26%, respectively). Interestingly, by Day 90 postpartum, despite some latent differences in metabolic profiles between groups, no evidence for a deleterious effect of lactation on the ability of the uterus to support conceptus elongation was observed.

3. Conceptus development in dairy cows

After fertilization of the oocyte in the oviduct, the resulting embryo is transported toward the uterus as it undergoes the first mitotic cleavage divisions. The bovine embryo enters the uterus at about the 16-cell stage on approximately Day 4 of pregnancy. Subsequently, it forms a compact ball of cells referred to as a morula in which cell-to-cell tight junctions are first established. By Day 7, the embryo becomes a blastocyst consisting of an inner cell mass which, after further differentiation, gives rise to the embryo, and the trophectoderm, which forms the placenta. After hatching from the zona pellucida on Days 9 to 10, the spherical blastocyst continues to grow and change its morphology. Conceptus elongation involves transitions from a spherical blastocyst on Day 7 of gestation, through ovoid (Days 12–13), tubular (Days 14–15), and finally filamentous forms around Days 16 to 17 [53]. After Day 19, the

fully elongated conceptus begins implantation with firm apposition and attachment of the trophectoderm to endometrial luminal epithelium. During elongation, the conceptus increases in size more than 1000-fold [54,55] associated with an increase in protein content [55,56].

Up to the blastocyst stage, the embryo is somewhat autonomous and does not need contact with the environment of the maternal reproductive tract, confirmed by the fact that blastocysts can be successfully developed *in vitro* in large numbers using IVF technology. Nonetheless, the maternal environment does modify the embryo during this time as evidenced by the positive effects of *in vivo* culture on various embryo quality parameters [57]. In contrast, normal development of the posthatching and preimplantation conceptus is entirely maternally driven, dependent on substances present in the uterine lumen, termed histotroph. These secretions which derive from the endometrium, particularly the uterine glands, are essential for growth and development of the conceptus. Normal conceptus elongation does not occur *in vitro* [58–60], and the experimentally induced absence of uterine glands *in vivo* results in a failure of blastocysts to elongate after ET [61,62]. While the importance of endometrial receptivity and gene expression during the pre- and peri-implantation period is well established, as mentioned earlier, there is evidence that the developing embryo can alter the endometrial transcriptome [63,64]. Thus, proper communication between the conceptus and endometrium is vital for pregnancy establishment.

Progesterone from the corpus luteum acts indirectly *via* the endometrium to stimulate embryonic growth and conceptus elongation [49,65]. Earlier studies in ewes [66,67] and cows [68] suggested that maternal progesterone regulates early conceptus growth and development. More recent studies have confirmed those findings and begun to unravel the underlying biology [46,47].

Numerous studies have reported a wide variation in length between conceptuses recovered on the same day, whether due to experimental conditions such as embryo source or due to altered progesterone concentrations. For example, one consistent observation from the multiple ET studies we have carried out, involving the transfer of 10 to 20 Day-7 blastocysts to the uterus of synchronized recipients and subsequent recovery, is the variation in conceptus size on Day 14, even among those recovered from the same uterus. This would suggest that factors intrinsic to the blastocysts transferred regulate, at least in part, development and would be consistent with the hypothesis that the quality of the oocyte regulates developmental competence [57]. It has been estimated that approximately one-third of viable blastocysts on Day 6 of development fail to elongate and maintain the pregnancy by Day 28 of gestation [8].

A considerable volume of data exist in the literature on global gene expression in early bovine embryos, particularly at the blastocyst stage, no doubt partly due to the ease with which such embryos can be recovered *in vivo* or be generated *in vitro*. Furthermore, most research conducted to investigate the period of conceptus elongation in cattle has focused on the biology of endometrium. In contrast, relatively few such studies have focused on the elongating conceptus [69–72] with only a small number focused specifically on conceptuses from dairy cows [73,74].

Thompson et al. [75] characterized postpartum metabolic and hormonal differences between nonlactating and lactating dairy cows and evaluated lactation and pregnancy effects on endometrium and conceptus expression of selected genes and characterized associations between conceptus and endometrial expression of genes in early pregnancy (Day 17). Lactating cows had greater plasma concentrations of β-hydroxybutyrate and blood urea N and lower concentrations of glucose and progesterone compared with nonlactating cows. Insulin-like growth factor 1 was lower for lactating cows and was greater for cows subsequently classified pregnant compared with cyclic. Using tissues from the same group of cows, Cerri et al. [76] determined effects of lactation and pregnancy on endometrial gene expression on Day 17 of the estrous cycle and pregnancy. In total, 210 genes were differentially regulated by lactation (136 downregulated and 74 upregulated), and 702 genes were differentially regulated by pregnancy (407 downregulated and 295 upregulated). The interaction effect of pregnancy and lactation affected 61 genes. Genes upregulated and downregulated in pregnant cows were associated with several gene ontology terms, such as defense response and interferon regulatory factor, cell adhesion, and extracellular matrix. Several genes upregulated by lactation, such as *IGHG1*, *IGLL1*, *IGK*, and *TRD*, were related to immune function. Developmental genes related to limb and neural development and glucose homeostasis (e.g., *DKK1*, *RELN*, *PDK4*) were downregulated by lactation. Reduction in expression of *DKK1*, e.g., could potentially be deleterious for the embryo as this molecule has been implicated in the regulation of embryo competence to develop to term [77].

Valour et al. [78] analyzed the change in gene expression related to dam physiological status in Day-18 embryos from growing heifers (GH), early-lactating cows (ELC), and late-lactating cows (LLC). Embryo metabolism was greatly affected by dam physiological status when GH were compared with ELC and GH with LLC but to a lesser extent when ELC was compared with LLC. Genes involved in glucose, pyruvate, and acetate utilization were upregulated in GH versus ELC conceptuses (e.g., *SLC2A1*, *PC*, *ACSS2*, *ACSS3*). This was also true for the pentose pathway (*PGD, TKT*), which is involved in synthesis of ribose precursors of RNA and DNA. The pathways involved in lipid synthesis were also upregulated in GH versus ELC. Despite similar morphological development, the molecular characteristics of the heifer embryos were consistently different from those of the cows.

Recently, we examined the effect of lactation on the conceptus transcriptome by transferring single embryos derived by the superovulation of nulliparous heifers to the uteri of synchronized postpartum dry and lactating Holstein cows [73]. To isolate the effect of the uterine environment and avoid confounding issues of the cow's own oocyte, we transferred single high-quality embryos recovered from nulliparous heifers to both groups of cows. Conceptuses derived from heifers artificially inseminated to a synchronized cycle were used as a

control. Results of RNA sequencing analysis of the conceptuses revealed no differences in gene expression patterns for conceptuses recovered from nonlactating cows compared to heifers or nonlactating cows versus lactating heifers, consistent with the findings of Valour et al. [78]. The transcripts programmed cell death 4 (neoplastic transformation inhibitor: *PDCD4*); pecanex-like protein 1 (*LOC101908863*); jagged 1 (*JAG1*); CDC42 effector protein (Rho GTPase binding) 4 (*CDC42EP4*); signal-induced proliferation-associated 1 like 3 (*SIPA1L3*); and Rho guanine nucleotide exchange factor (GEF) 12 (*ARHGEF12*) were higher in conceptuses recovered from nonlactating cows, whereas the mRNA levels of TSR1, 20S rRNA accumulation, homolog (*S. cerevisiae*) (*TSR1*), and solute carrier family 10 (sodium/bile acid cotransporter), member 1 (*SLC10A1*) were lower in conceptuses recovered from nonlactating cows compared to those recovered from heifers. In contrast, 100 differentially expressed genes were found with higher and 169 with lower transcript abundance in conceptuses recovered from lactating cows compared to those recovered from heifers. The transcript levels of Dickkopf WNT signaling pathway inhibitor 4 (*DKK4*); pregnancy-associated glycoprotein 1-like (*LOC101903717*); uncharacterized LOC101906588 (*LOC101906588*); phosphoglucomutase 2-like 1 (*PGM2L1*); interferon regulatory factor 2 (*IRF2*); guanine deaminase (*GDA*); cytochrome P450, family 39, subfamily A, polypeptide 1 (*CYP39A1*); short stature homeobox 2 (*SHOX2*); MX dynamin-like GTPase 2 (*MX2*); and mucin 4, cell surface associated (*MUC4*) were all higher in trophoblast cells of conceptuses recovered from lactating cows compared to heifers, whereas those of wingless-type MMTV integration site family, member 2B (*WNT2B*); heat shock 70-kDa protein 6 (HSP70 B′) (*HSPA6*); neuronal guanine nucleotide exchange factor (*NGEF*); pleckstrin homology domain containing, family F (with FYVE domain) member 1 (*PLEKHF1*); methylenetetrahydrofolate dehydrogenase (NADP + dependent) 1-like (*MTHFD1L*); keratin 18 (*KRT18*); neurogranin (protein kinase C substrate, RC3) (*NRGN*); sodium channel, voltage-gated, type III, alpha subunit (*SCN3A*); tubulointerstitial nephritis antigen (*TINAG*); and uncharacterized LOC104973964 (*LOC104973964*) were decreased in the conceptuses recovered from lactating cows to the greatest extent on the basis of fold change difference.

Analysis of 18 amino acids from the uterine luminal fluid (ULF) of these animals revealed significantly lower concentrations of small neutral amino acids (alanine, glycine, serine, and threonine), a basic amino acid (arginine), two large neutral amino acids (leucine and valine) in ULF of heifers on Day 19 compared to both nonlactating and lactating cows, whereas three amino acids (glutamic acid, glutamine, and lysine) were lowest in heifers compared to nonlactating and lactating cows, but concentrations were also significantly higher in lactating cows compared to nonlactating cows. Interestingly, no differences in expression values for amino acid transporters were identified in either the conceptus of intercaruncular endometrium of these animals. Results suggest that the differences observed are due to the maternal environment given that embryos were derived from the same pool before transfer although whether they represent plasticity of the conceptus or have consequences for pregnancy outcome require further investigation.

4. Endometrial function in dairy cows

Given what was mentioned previously about the dependency of the conceptus on the maternal uterine environment for elongation to occur, it is appropriate to briefly discuss the uterus itself, although as with the conceptus, data from dairy cows are relatively scarce. The uterus plays a central role among the reproductive tissues in the context of early embryo–maternal communication, and a successful pregnancy depends on a complex series of endometrial molecular and cellular events. The transcriptome of the endometrium is influenced by a number of factors including the periovulatory endocrine milieu [79], postovulatory progesterone concentrations [45–47,80], uterine disease [81–83], genetic merit for fertility [84,85], and presence of a conceptus [86,87]. However, although the endometrial transcriptome signature can influence conceptus development, it also is reflective of the quality or type of embryo present; different types of embryos elicit different responses which may be predictive of subsequent fate [63,64,88].

Mesquita et al. [79] reported that the periovulatory endocrine milieu regulates the endometrial transcriptome in cattle and seems to determine the transition from a proliferation permissive to a biosynthetic and metabolically active endometrial phenotype, which may be associated with the preparation of an optimally receptive uterine environment. Moran et al. [84] sequenced the transcriptome of endometrial biopsies collected on Day 7 of the estrous cycle from cows of high and low fertility, as described previously [40,41]. Significant differential expression of 403 genes between high- and low-fertility cows was found. A novel network-based functional analysis highlighted 123 genes from three physiologically relevant networks of the endometrium: (1) actin and cytoskeletal components; (2) immune function; and (3) ion transportation, providing molecular evidence for an association between gene expression in the uterine environment and genetic merit for fertility in dairy cows.

A significant body of literature has demonstrated large effects of disease on fertility in dairy cows [89–91]. Amongst recent studies, Oguejiofor et al. [83] described the dysregulation of endometrial immune response to bacterial lipopolysaccharide (LPS). After exposure of primary cultures of mixed bovine epithelial and stromal endometrial cells to LPS for 6 hours, approximately 30% of the 1006 genes altered by LPS were classified as being involved in immune response. Cytokines and chemokines (*IL1A, CX3CL1, CXCL2*, and *CCL5*), interferon-stimulated genes (*RSAD2, MX2, OAS1, ISG15*, and *BST2*), and the acute phase molecule *SAA3* were the most upregulated genes. Additionally, many genes involved in endometrial response to the conceptus in early pregnancy were also altered by LPS, suggesting one mechanism whereby an ongoing response to infection may interfere with the establishment of pregnancy. Ribeiro et al. [74] characterized the impact of inflammatory uterine (retained placenta and metritis) and

nonuterine (mastitis, lameness, digestive and respiratory problems) diseases before breeding on developmental biology and reproduction in cows. Inflammatory disease before breeding negatively impacted oocyte fertilization, development to morula, and impaired early conceptus development to elongation stages and secretion of IFN-τ. Diseases caused inflammation-like changes in transcriptome of conceptus cells, increased risk of pregnancy loss, and reduced pregnancy or calving per breeding. Occurrence of disease at preantral or at antral stages of ovulatory follicle development had similar detrimental effects on pregnancy outcome indicating carryover effects of diseases might last longer than 4 months. The authors concluded that reduced oocyte competence is a likely reason for carryover effects of diseases on developmental biology, but impaired uterine environment was also implicated.

4.1. Conceptus-induced effects on the endometrium

We recently examined conceptus-derived proteins, in addition to IFNT, that may facilitate pregnancy recognition in cattle [92]. Analysis of the protein content of the ULF from cyclic heifers on Day 16 by nano-liquid chromatography tandem mass spectrometry identified 334 proteins. Comparison of these data with 299 proteins identified in the ULF of pregnant heifers on Day 16 identified 85 proteins only present in the ULF of pregnant heifers. Analysis of Day-16 conceptus-conditioned culture medium recovered after 6- and 24-hour incubation *in vitro* revealed the presence of 1005 proteins of which 30 were unique to ULF from Day-16 pregnant heifers. Of these 30 proteins, 12 had mRNA expression values at least twofold higher in abundance in the conceptus compared to the endometrium (*ARPC5L, CAPG, CKMT1, CSTB, HSPA8, HSPE1, LGALS3, MSN, NUTF2, P4HB, PRKAR2A, TKT*) as determined by RNA sequencing. In addition, genes that have a significant biological interaction with the proteins (*ACO2, CKMT1, CSTB, EEF2, GDI1, GLB1, GPLD1, HNRNPA1, HNRNPA2B1, HNRNPF, HSPA8, HSPE1, IDH2, KRT75, LGALS3, MSN, NUTF2, P4HB, PRKAR2A, PSMA4, PSMB5, PSMC4, SERPINA3, TKT*) were differentially expressed in the endometrium of pregnant compared to cyclic heifers during the pregnancy recognition period (Days 16–18). These proteins that were unique to ULF from pregnant heifers and produced by short-term *in vitro* cultured Day-16 conceptuses could potentially be involved in facilitating the interactions between the conceptus and the endometrium during the pregnancy recognition period.

5. Conclusion

Subfertility is a multifactorial issue resulting as a consequence of insults at various points along the developmental axis. Evidence exists implicating the oocyte, the embryo, and the reproductive tract in subfertility. Improved understanding of the developmental biology involved in conceptus development and uterine receptivity in cattle may contribute for the development of strategies to minimize embryonic losses and improve reproductive efficiency in cattle.

Acknowledgments

The authors acknowledge support from Science Foundation Ireland (grant number 13/IA/1983) and the European Union Seventh Framework Programme FP7/2007-2013 under grant agreement n° 312097 ('FECUND').

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